IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : **09/992,957**

Applicants : **Hans Herweijer et al.**

Filed : **11/13/2001**

Art Unit : **1636**

Examiner : **Sullivan, Daniel M.**

Docket No. : **Mirus.025.01**

For: **Methods for Genetic Immunization**

Commissioner of Patents
PO Box 1450
Alexandria, VA 2231-1450

DECLARATION UNDER 37 C.F.R. §1.132

Dear Commissioner:

I, Mary Kay Bates, hereby declare as follows:

1. I am an inventor of the captioned application.
2. I submit with this Declaration and Response further experimental material (attached) illustrating: genetic immunization in mice, rats, and rabbits via tail vein injection or limb vein injection of nucleic acid. The material demonstrates that the process is effective in a majority of individual mammals injected.
3. The material is consistent with the specification as filed and only methods described in the specification have been used. No new matter was used in the experiments.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Mary Kay Bates 11-23-05

Mary Kay Bates Date

GENETIC IMMUNIZATION BY HYDRODYNAMIC TAIL VEIN (HTV) AND HYDRODYNAMIC LIMB VEIN (HLV) GENE DELIVERY

A typical objective of genetic immunization is the isolation of polyclonal antibody containing sera or antibody-producing B cells, which can be used to generate monoclonal antibody (MoAb) producing cell lines (either by immortalization or by fusion with myeloma cells to form hybridomas).

For immunizations, six-week old ICR mice (~20 g), six-to-seven week old (~125 g) Sprague-Dawley rats and two-month old (~2.2 kg) New Zealand White rabbits were used. For hydrodynamic tail vein delivery in mouse, plasmid DNA was diluted in Ringers' solution and a volume equal to 10% of the body weight was injected into the lateral tail vein in 6 to 7 seconds with the maximum volume to be delivered capped at 3.0 ml (mice $\geq$ 30 gram body weight). A similar procedure was used for hydrodynamic tail vein delivery in rats, with a delivery time of 18-22 seconds and a maximum volume of 20 ml (rats $\geq$ 200 g). For hydrodynamic limb vein gene delivery procedures, a latex tourniquet was wrapped around the upper hind limb to block blood flow and tightened in place with a hemostat. A small incision was made to expose the distal saphenous vein and a catheter was inserted into the vein. A syringe pump was used to deliver the pDNA containing saline solution (0.05 ml/g in mice; 0.08 ml/g in rats; 33 ml/kg in rabbits). The needle was retracted and the tourniquet released two minutes after pDNA delivery. Bleeding was controlled with pressure and a hemostatic sponge. The incisions were closed with 4-0 Vicryl sutures. For direct intramuscular injections in mice, 50 μg pDNA was injected in 100 μl saline solution into the quadriceps, using a 30 gauge needle in 2-3 seconds. Blood samples were taken via retro-orbital bleed from rodents; rabbits were bled via the marginal ear vein.

Genetic immunization by hydrodynamic tail vein plasmid DNA delivery in mice:
Mice were injected 5 times with 50 μg pCI-Luc (delivered on days 0, 14, 21, 28 and 35), either by HTV. Sera were collected on day 35 and 42 for anti-luciferase Ab quantitation by ELISA. On day 35, the average level of anti-luciferase Abs in HTV immunized mice

was 334.3 ± 122.2 μg/ml (5 mice). Antibody levels approximately doubled by day 42. All 5 mice exhibited an immune response as determined by antigen-specific antibody production. An immune response was generated after a single injection using an expression vector with the ubiquitin promoter, which generates sustained expression in the liver. Similarly, antibody production was observed in mice following HTV gene delivery of human CD4 and canine dystrophin expression vectors. In 25 mice injected with hCD4 expression vector, all 25 mice generated anti-hCD4 specific antibodies.

Of 21 different antigens tested (6 or more mice per antigen), HTV genetic immunization induced antibody production against 14 of the antigens. For the 14 antigens that induced antibody production, this immune response was observed in 272 out of 276 mice (including 25 out of 25 mice immunized with hCD4 and 189 out of 189 mice immunized with Luciferase). Only antibody production was measured. For animals in which no antibodies were detected by ELISA or Western blot analyses, no other tests were performed to detect an immune response, though other forms of immune response were possible.

Genetic immunization by hydrodynamic limb vein plasmid DNA delivery in mice: Intravenous pDNA injection into veins of limbs temporarily isolated by a tourniquet results in very high gene transfer to skeletal muscle cells. This hydrodynamic limb vein procedure is readily applied in small rodents (mouse, rat and rabbit) and larger mammals (dog and primate) with similar transfection efficiencies. Mice were injected with 1, 2, 3 or 4 doses of pCI-Luc. Groups of mice received repeat doses of pCI-Luc in the same limb or in alternate limbs. ELISA results demonstrated that very high levels of anti-luciferase Abs were generated with only two HLV gene deliveries in all mice tested. Injection into a single limb or to alternating limbs resulted in similar Ab levels.

Mice were injected via HLV four times (day 0, 14, 21 and 28) with 50 μg of pDNA vectors expressing the secreted proteins hepatitis B virus e antigen (HBVe) or rat erythropoietin (rEPO). Western blot analysis of HBVe using injected mouse antisera showed detection of the unmodified C antigen precursor (24 kDa) and the mature e

antigen (16 kDa). Western blot analysis of rEPO using injected mouse antisera showed detection of the protein of predicted size, 34 kDa.

Mice immunized via HLV generated specific, high titer Abs to 83% of expressed proteins of tested antigens (5 of 6 antigens; luciferase, human gp100, rEPO, HBVe, hVEGF165). Thus, HLV gene delivery is an effective method for generating Abs to expressed proteins in mice. For antigens which generated an antibody production, all mice injected developed this immune response. As before, the presence of other immune responses was not tested. For three antigens for which no antibody production was detected following immunization via tail vein injection, antibody production was observed when the same plasmid was delivered by limb vein injection.

Genetic immunization by hydrodynamic limb vein plasmid DNA delivery in rats:
Rats were injected 4 times with 500 μg of the luciferase expression vector pCI-Luc (day 0, 14, 21, 28). Sera were collected at several time points after immunization and analyzed for anti-luciferase Abs by ELISA. All mice injected produced luciferase antigen-specific antibodies.

Genetic immunization by hydrodynamic limb vein plasmid DNA delivery in rabbits:
Rabbits were injected with 1 mg/kg body weight pCI-Luc, either 2 (day 0, 14) or 3 times (day 0, 14, 21), via the hind limb saphenous vein (two rabbits per group). Individual rabbit anti-luciferase Ab levels were measured by ELISA on day 35 and 42. High levels of anti-luciferase Abs were present in the sera on day 35 after either two injections (100.4 and 585.0 μg/ml) or three injections (286.8 and 345.7 μg/ml). Titers were approximately double on day 42. 100% of rabbits (16 out of 16) injected by HLV gene delivery injection generated high titer, specific Abs.

Conclusion
67% of antigens tested resulted in successful antigen-specific antibody production when the expression vector was delivered by hydrodynamic tail vein injection in mice. For antigens that induced an immune response, the response was observed in nearly all mice

injected (272 of 276). The process was also shown to be effective in rats. Injection into limb yielded an even higher success rate: 92% of the antigen expression vectors delivered by HLV induced antibody production. This method was shown to be successful in mice, rats and rabbits.

Three of the antigens that failed to elicit antibody production following HTV genetic immunization were predicted to be secreted proteins. For these three antigens, HLV genetic immunization was successful in eliciting antibody production. Thus, the site of expression may influence the properties of the antigen in generating an antibody production immune response.

Additional Examples:

Comparison of alternate delivery routes for genetic immunization of mice. The luciferase expression vector pMIR48 was administered to ICR mice by each of three methods: intramuscular and intravascular delivery of naked pDNA, and intravascular delivery of pDNA particles (5 animal per group). For direct intramuscular injections in the quadriceps, 50 μg plasmid DNA in 100 μl saline was injected. For intravascular delivery via hydrodynamic tail vein injection, 50 μg plasmid DNA in 1 ml Ringer's solution per 10 g mouse body weight was injected in about 7 seconds. For low-pressure tail vein injection, 50 μg plasmid DNA was complexed with the polycation polyethylenimine (PEI) and recharged with the polyanion polyacrylic acid (PAA) at a ratio of 1:6:1 (wt:wt:wt) in a volume of 50 μl. Mice were injected on days 0, 14, 21 and 28. To quantitate anti-luciferase antibody titers, sequential serum samples were taken before the initial (prime) injection and 7 days after each injection and analyzed by standard ELISA test. A standard curve was generated using a commercially available anti-luciferase antibody. The results (FIG. 2) demonstrate that increased pressure intravascular delivery of naked pDNA resulted in higher titers and more rapid induction of anti-luciferase antibodies than the more conventional injection into skeletal muscle. Dose response experiments (not shown) have indicated that after two booster injections with 10 μg pDNA delivered IV resulted in higher titers than the highest dose delivered IM (100 μg).

PEI/PAA particles are better than IM injection, even though the final titers are lower than after IV immunization. Of the mice immunized by intravascular tail vein injection of DNA, all animals developed an immune response.

Generation of antibodies in mouse to human dystrophin. An anti-human dystrophin antibody was generated in ICR mice by genetic immunization. The mice were primed and boosted by high pressure tail vein delivery of 100 μg of a human dystrophin expression cassette (2 boosts at 2 and 3 weeks after the prime). Sera were obtained 3 days after the second boost and used to stain for human dystrophin expression in *mdx* (dystrophin deficient) mice previously injected with 10 μg of the same expression vector (IM). Immunohistochemistry with the antisera showed a the presence of myofibers expressing human dystrophin in a typical dystrophin staining pattern. These results were identical to those obtained with commercially available anti-human dystrophin antibodies. Thus intravascular genetic immunization can result in the generation of antibodies against clinically relevant target proteins with titers are sufficient to be used for immunohistochemistry. The antisera was further shown to cross react with the mouse dystrophin in ICR (dystrophin positive mice). Dystrophin staining in ICR mouse with the antisera is shown in FIG 3. The left panel of FIG. 3 shows mouse skeletal muscle stained with the anti-human dystrophin polyclonal antisera using a labeled anti-mouse IgG secondary antibody for fluorescence detection. The right panel shows mouse skeletal muscle stained with a commercially available anti-human dystrophin monoclonal antibody that does not cross react with mouse dystrophin.

Comparison of intravascular genetic immunization to standard intramuscular injection. 50 or 100 μg of DNA encoding firefly luciferase were injected into mice 4 times as described above. The first injection, the prime injection, occurred at day 0. Subsequent injections occurred on days 14, 21 and 28. Antisera from mice were tested at various times before, during and after immunization. As shown in the table, genetic immunization by intravascular delivery of polynucleotide resulted in higher antigen-specific antibody titers than did intramuscular injection of polynucleotide. Similar results were observed in animals which received injections in which the DNA was in: a)

standard Ringers' solution, b) standard Ringers' solution + 5% mannitol or c) 50% standard Ringers' solution/50% saline + 3.75% mannitol.

Levels of anti-luciferase antibody titers (μg/ml antibody concentration) generated by intravascular tail vein injection versus direct intramuscular injection. Levels shown are averages of five mice per group. All animals responded and developed an anti-luciferase antibodies.

	Intravascular		Intramuscular	
day	50 μg DNA	100 μg DNA	50 μg DNA	100 μg DNA
0	0.05 ± 0.03	0.04 ± 0.01	0.09 ± 0.05	0.04 ± 0.01
7	0.12 ± 0.04	0.23 ± 0.12	0.07 ± 0.02	0.06 ± 0.01
20	17.7 ± 20.3	20.3 ± 15.6	1.06 ± 1.70	1.85 ± 2.80
27	36.8 ± 25.9	79.2 ± 28.9	1.90 ± 2.42	5.98 ± 4.25
35	334 ± 244	344 ± 234	6.62 ± 9.57	3.73 ± 4.51
42	668 ± 366	926 ± 229	14.3 ± 21.8	17.4 ± 16.8

Generation of antibodies to mammalian antigens in mice. Mice were immunized, as described above for tail vein injection, with polynucleotides encoding either a truncated human CD 4 protein or canine dystrophin. CD4 represent a membrane-bound antigen and dystrophin represents an intracellular antigen. For both, mice were immunized by the intravascular tail vein procedure described above. 50 μg plasmid DNA injected into the tail vein on days 0, 14, 21 and 28. Blots, containing extracts from cells expressing either the immunizing antigen (+ lanes) or a control protein (− lanes) were probed with sera sampled on day 35. Sera were diluted 1:100. The CD4 protein has a predicted protein size of approximately 46 kD and the canine dystrophin has a predicted protein size of 425 kD. FIG. 4 shows that each of the mice produced antigen-specific antibodies. The experiment was repeated with similar results: all animals developed antibodies to the protein encoded by the injected nucleic acid.

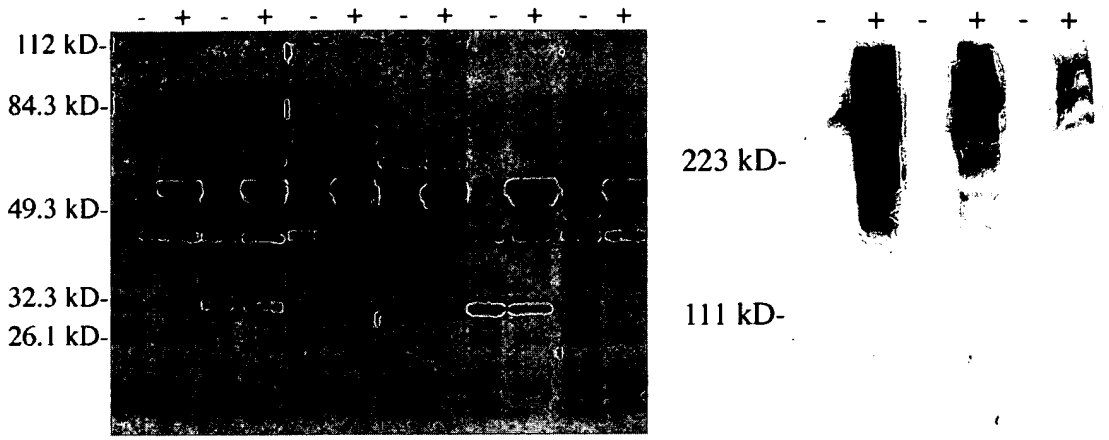

FIG. 4. Western blots illustrating presence of antibodies to mammalian proteins in mice immunized with polynucleotides encoding human CD4 or canine dystrophin. The left panel shows detection of antigen using antisera from mice injected with CD4 encoding polynucleotide (predicted size 46 kD). The right panel shows detection of antigen using antisera from mice injected with dystrophin encoding polynucleotide (predicted size 425 kD). Each set of two lanes (− and +) represents serum from an individual mouse (− lane = cell extract lacking antigen; + lane = cell extract containing antigen).

Hybridoma Fusion Using Splenocytes from Mice Immunized via Intravascular Delivery of a Plasmid. Six mice were immunized with pMIR167 encoding human Ki67, a chromatin-binding protein, via four injections into tail vein as described above. Analysis of the mouse antisera showed very strong signal (results not shown). Animal were given a fifth immunization and day 105 and spleens were harvested four days later. Splenocytes were frozen and processed for hybridoma fusion using methods standard in the art. 46 clones were isolated that presented typical Ki-67 pattern in immuno-cytochemical staining. None of the supernatants cross-reacted with mouse. Two cross-reacted with rat. Almost all cross-reacted with monkey Ki67. Five of these culture supernatants, along with a commercially available anti-Ki67 antibody are shown detecting Ki67 in HeLa cells in FIG. 5. All six animals injected yielded antibody producing immune cells.

Antibodies generated via intravascular genetic immunization maintain high titers over long-term. Four mice were immunized via intravascular tail vein delivery of

polynucleotides as described above. Mice were injected with 10 μg pMIR48 on days 0, 14, 21 and 28. High titer was observed in all four mice at day 48 as tested by ELISA, three weeks after the last boost. This level was maintained for at least another 32 days.

day	anti-luciferase antibody titer (μg Ab/ml serum)
0	0.01
13	0.02
20	0.39
27	5.40
34	8.76
41	16.5
48	46.9
76	48.5

Intravascular genetic immunization in rats. Rats were genetically immunized via intravascular delivery of polynucleotide as described for mouse immunization. 500 μg pMIR48 in 20 ml was injected into the tail vein of rats in 20 sec. Rats were injected on days 0, 14, 21 and 28. On day 35 animals were bled and the sera were tested for the presence of anti-luciferase antibodies by Western blot. The data in FIG. 6 shows luciferase-specific antibodies were present in the injected rats (− lane = cell extract lacking antigen; + lane = cell extract containing antigen), demonstrating the application of intravascular genetic immunization in larger rodents. Four of four rats immunized in this way produced antigen-specific antibodies.

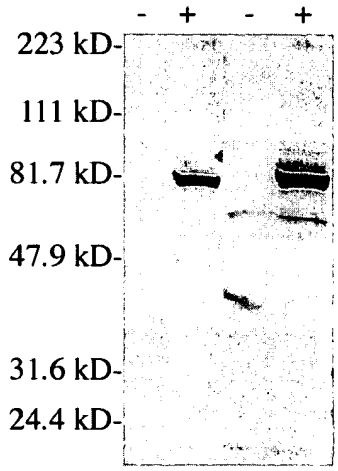

FIG. 6. Western blot showing induction of luciferase-specific antibodies in rats following intravascular genetic immunization. The blot contains cell extracts for COS7 cells either expressing a control protein (− lanes) or luciferase (+ lanes). Rat antisera were used at a 1:100 dilution. Secondary anti-rat HRP antibody (Sigma) was used at a 1:5000 dilution.

Induction of immune response in mice following intravenous delivery of a polynucleotide: Four mice were injected on days 0, 14 and 21 with a plasmid encoding the firefly luciferase gene under control of the cytomegalovirus promoter (pMIR48). For each injection, a solution containing the plasmid was inserted into lumen of the saphenous vein animals as described in U.S. Application No. 10/855,175 (incorporated herein by reference) and as follows: A latex tourniquet was wrapped around the upper hind limb just above the quadriceps and tightened into place with a hemostat to block blood flow to and from the leg. A small incision was made to expose the distal portion of the great (or medial) saphenous vein. A 30 gauge needle catheter was inserted into the distal vein and advanced so that the tip of the needle was positioned just above the knee in an antegrade orientation. A syringe pump was used to inject an efflux enhancer solution (42 μg papaverine in 0.25 ml saline) at a flow rate of 4.5 ml/min followed 1 – 5 min later by injection of 1.0 ml saline containing 10 μg pDNA per injection at a flow rate of 4.5 ml/min. The solution was injected in the direction of normal blood flow through the vein. Two minutes after injection, the tourniquet was removed and bleeding was

controlled with pressure and a hemostatic sponge. The incision was closed with 4-0 Vicryl suture. The procedure was completed in ~10 min.

As controls, two mice were immunized via plasmid delivery to the liver using tail vein injections (retrograde injection). Mice received injections on the same day as indicated above. For the tail vein injections, 10 μg plasmid DNA in 2.5 ml Ringer's solution per injection was injected into the tail vein using a 27 gauge needle. The entire volume was delivered in less than 10 sec.

To monitor induction of an immune reaction to luciferase, the animals were bled on days 0, 13, 20, 27, 34, 41 and 48. The blood was allowed to clot and the sample was centrifuged to recover the sera. Sera were analyzed for the presence of antibodies to luciferase using an ELISA, as follows: 96-well plates were coated with a recombinant luciferase protein (Promega, Madison, WI) by incubation of 100 μl 2 μg/ml protein in 0.1 M carbonated buffer per well. Plates were incubated overnight at 4°C, then washed three times with PBS containing 0.05% Tween 20. Wells are blocked with 200 μl PBS + 1% non-fat dried milk for 1.5 h at RT and washed three times as above. Mouse sera were diluted in PBS + 1% milk. 100 μl diluted sera were added to wells in duplicate and incubated 1.5 h at RT. The plates were washed three times as above. 100 μl anti-mouse polyvalent antibody conjugated to horseradish peroxidase (Sigma, St. Louis, MO) diluted 1:20,000 in the PBS + 1% milk buffer was added to each well. The plates are washed five times as above. 100 μl tetramethylbenzidine (Sigma) was added to each well and the samples were allowed to develop. The reaction was stopped by addition of 100 μl 1.0 M H_2SO_4 per well and the absorbance was read at 450 nm. A standard curve was generated using a goat anti-luciferase horseradish peroxidase conjugate (Sigma). The results are shown in the table below. The presence of anti-luciferase antibodies in the mouse sera indicates successful induction of an immune response.

Antibody concentration (μg/ml) in mice genetically immunized via injection of plasmid DNA into either tail vein or saphenous vein. All mice injected, tail vein injection and saphenous vein injection, developed an immune response

day	tail vein	saphenous vein
0	0.13	0.09
13	0.06	2.03
20	1.72	51.6
27	47.1	175
34	106	471
41	174	332
48	235	393

Intravascular genetic immunization via injection into limb vein. Four ~150 g Sprague-Dawley rats per group were immunized with 500 μg pMIR48. Group 1 animals were immunized by delivery of antigen-encoding polynucleotide via saphenous vein injection Plasmid DNA in 3 ml of normal saline solution (NSS) was used for each injection. Blood flow to and from the limb was restricted just prior to and during the injection, and for 2 min post-injection by placing a tourniquet around the upper leg (just proximal to/or partially over the quadriceps muscle group). The solution was injected into the great saphenous vein of the distal hind limb at a rate of 3 ml per ~20 seconds (10 ml/min). The intravenous injections were performed in an anterograde direction (i.e., with the blood flow) via a needle catheter connected to a programmable Harvard PHD 2000 syringe pump (Harvard Instruments). Group 2 animals received immunization via hydrodynamic delivery of polynucleotide through the tail vein. Immunizations occurred on days 0, 13, 20, 20, 27, 25 and 42 and animals were bled on days 7, 20 and 28. Sera were separated and tested in a single ELISA.

Results are shown in μg/ml antibody concentration.

Day	Tail vein	Saphenous vein
0	0.15 ± 0.10	0.34 ± 0.28
13	0.26 ± 0.11	13.3 ± 19.8
20	0.31 ± 0.13	62.9 ± 75.3
27	0.42 ± 0.07	102 ± 102
35	0.75 ± 0.53	469 ± 308
42	0.61 ± 0.27	490 ± 370

Similar results were obtained with two other antigens.

Antibody generation via intravascular genetic immunization works in larger animals as well as mice, as demonstrated in rabbits. Four rabbits were injected on days 0, 14, 21 and 28 with a plasmids encoding the firefly luciferase gene under control of the cytomegalovirus promoter (pMIR48) and the ubiquitin C promoter and a hepatic control region for enhancement of long-term expression (pMIR68). Two animals also received a plasmid encoding murine interleukin 2 under control of the cytomegalovirus promoter (pMIR152).

For each injection, a solution containing the plasmid was inserted into the lumen of the saphenous vein as follows: A latex tourniquet was wrapped around the upper hind limb to block blood flow into and out of the leg and tightened into place with a hemostat. Injections were done into either the great or the small saphenous vein. A 23 gauge catheter was inserted, in antegrade orientation, into the lumen of the vein. A syringe pump was used to inject an efflux enhancer solution (1.0 mg papaverine in 6 ml) at a flow rate of 4-5 ml/min. One to five minutes later a solution containing plasmid DNA was injected through the catheter (1 mg/kg pMIR48 or pMIR68; 2 mg/kg pMIR152 in 18-44 ml saline, 14 ml/kg animal weight.) The solution was injected in 18-30 seconds (1-2 ml/sec). The volume of solution and rate of injection were varied depending on the weight of the rabbit. The solution was injected in the direction of normal blood flow through the vein. The tourniquet was removed two minutes after the injection. Bleeding from the incision and vein puncture was controlled with pressure and a hemostatic sponge. The incision was closed with 4-0 Braunamid suture. The procedure was completed in ~20 min.

To monitor induction of an immune reaction to luciferase in the animals, animals were bled via the ear vein. The presence of antibodies in the sera, indicating induction of an immune response, was determined by ELISA and Western blot. The results are shown in FIG. 7. The presence of anti-luciferase antibodies in the rabbit sera indicates successful induction of an immune response. These results demonstrate the applicability of intravascular genetic immunization in larger animals that can be used to produce

polyclonal antibodies on a larger scale. All six rabbits injected developed a good immune response.

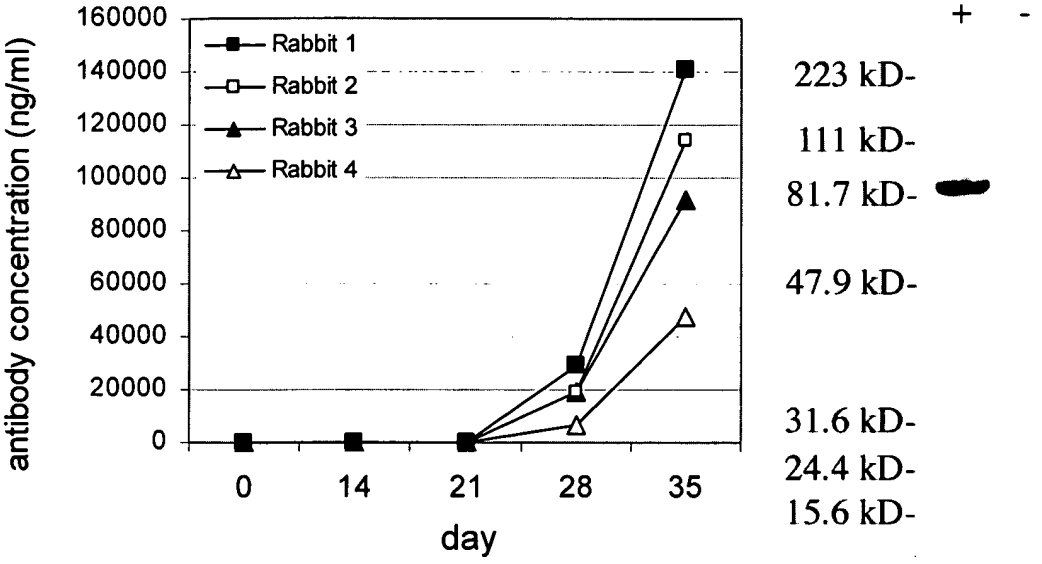

FIG. 7. Antibody production against luciferase protein by genetic immunization of rabbits limb vein injection of antigen expressing polynucleotides. The left panel shows time course of antibody expression detected via ELISA. The right panel shows a Western blot using serum from immunized rabbit. The blot contained cell extracts for COS7 cells either expressing a control protein (– lane) or luciferase (+ lane). Rat antisera were used at a 1:100 dilution. Secondary anti-rat HRP antibody (Sigma) was used at a 1:5000 dilution.

Comparison: Immunogen expression vectors. To test if sustained expression of the antigen may require fewer boosts, we compared genetic immunization of two luciferase vectors: pMIR48 (CMV promoter) and pMIR68 (ubiquitin C promoter). Previous experiments have shown that pMIR68 generates stable luciferase expression for many months at a level about ten-fold below CMV-driven peak levels. FIG. 8 shows the result of antibody induction following hydrodynamic tail vein delivery of 10 μg pMIR48 or pMIR68 alone or a combination of the two (5 μg each). We also compared the effect of 2 boosts versus no boost. A single delivery of pMIR48 did not result in significant antibody titers by day 42, whereas pMIR68 immunized mice did show significant levels of anti-luciferase antibodies. Boosting increased antibody levels significantly, and no differences between the different plasmid DNA groups were observed.

Enhanced immune response by co-delivery of cytokine expression vectors. The immunomodulator Flt3-Ligand (Flt3-L) acts on $CD34^+$ progenitor cells and results in increases in DC and NK cells. Intravascular delivery of a CMV promoter-driven Flt3-L vector into ICR mice via tail vein injection was performed to determine the effects of delivery of the Flt3-L gene. Different levels of the expression vector were injected and the number and composition of spleen cells was analyzed after 10 days. Delivery of 10 μg murine Flt3-Ligand pDNA increased the total splenocyte count 3.8 fold (260 million cells per spleen for Flt3-L treated mice compared to 68 million cells per spleen for control mice). Furthermore, the splenocytes demonstrated an increase in the percentage of CD11c+ dendritic cells. 2.3% CD11c+ splenocytes were observed in control mice while 24.5% CD11c+ splenocytes were observed in mice receiving Flt3-L pDNA. A dose-dependent response in total number of splenocytes and CD11c+ cells was observed when delivering a range of 1-50 μg/mouse of Flt3-L pDNA.